Chemical Constituents of *Capparis tenera* Leaves (Postprint)

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Abstract

To investigate the chemical constituents in the leaves of the characteristic plant Champereia manillana var. longistaminea, this study employed silica gel column chromatography (CC), thin-layer chromatography (TLC), Sephadex LH-20 column chromatography, reversed-phase silica gel (RP-18) column chromatography, and high-performance liquid chromatography (HPLC) to isolate and purify the ethyl acetate fraction of the ethanol extract from the leaves of Champereia manillana var. longistaminea, yielding six monomeric compounds. The structures of these compounds were elucidated by nuclear magnetic resonance spectroscopy (NMR) and high-resolution electrospray ionization mass spectrometry (HR-ESI-MS) data, combined with comparison to literature data. The six compounds were identified as taraxerol (1), indole-3-carboxylic acid (2), (24R)-cycloartane-3β,24,25-triol (3), (24R,S)-3β-24,31-epoxy-24-methylcycloartane (4), 1-O-linoleoyl-3-O-β-D-galactopyranosyl-sn-glycerol (5), and long-chain alkyl glycerol monoester (6), among which compounds 1–6 were all isolated from this plant for the first time.

Full Text

Chemical Constituents from the Leaves of *Champereia manillana* var. *longistaminea*

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Abstract: To investigate the chemical constituents in the leaves of *Champereia manillana* var. *longistaminea*, the ethyl acetate fraction of the ethanol extract was isolated and purified using silica gel column chromatography (CC), thin-layer chromatography (TLC), dextran gel column chromatography (Sephadex LH-20), reversed-phase silica (RP-18) column chromatography, and high-performance liquid chromatography (HPLC). Six monomeric compounds were obtained, and their structures were identified by nuclear magnetic resonance spectroscopy (NMR) and high-resolution mass spectrometry (HR-ESI-MS) data in comparison with literature values. The six compounds were identified as taraxerol (1), indole-3-carboxylic acid (2), (24R)-cycloartane-3β,24,25-triol (3), (24R,S)-3β-24,31-epoxy-24-methylcycloartane (4), 1-O-linolenoyl-3-O-β-D-galactopyranosyl-sn-glycerol (5), and hyloglyceride (6). All compounds 1–6 were isolated from this plant for the first time.

Keywords: *Champereia manillana* var. *longistaminea*; extraction and separation; purification; chemical composition; structure identification

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Champereia manillana var. *longistaminea* belongs to the family Opiliaceae and genus *Champereia* Griff., a shrub or evergreen small tree that bears flowers and fruits directly on its stems, hence its common name. Also known as *Linwei mu* (Qin & Liu, 2010), it has various local names including "Tiancai shu" (Funing, Yunnan), "Weijing shu" or "Leigong cai" (Tianlin, Guangxi), and "Longxu cai" (Tiandong, Guangxi). This characteristic woody economic plant thrives in karst regions of China, primarily distributed in southeastern Yunnan and southwestern Guangxi, where it grows in dense valley forests or rock crevices (Du et al., 2018). The tender stems and leaves are rich in nutrients and bioactive substances, including vitamins, zinc, calcium, iron, and various pharmacologically active amino acids. Locally, the tender leaves are consumed as a vegetable to prevent cardiovascular and cerebrovascular diseases, hypertension, and diabetes, demonstrating significant edible and medicinal value (Zhu et al., 2018). Previous research has explored its wild resources, seedling cultivation techniques, biological characteristics, fruit features, and nutritional components (Yang, 2008; Wei et al., 2019; Wei et al., 2020), establishing its potential as an excellent economic plant. The distinctive flavor of *C. manillana* originates from its high content of glutamic and aspartic acids (Tang et al., 2020), while iditol, a sweet functional factor identified in its tender stems and leaves, can serve as a synthetic intermediate in the food and pharmaceutical industries (Liu & Xiao, 2009). Despite these promising applications, research on the plant' s chemical constituents and pharmacological effects remains largely unexplored, severely limiting its further development and utilization. To elucidate its pharmacological material basis and uncover additional medicinal benefits, this study investigated the chemical constituents from 95% ethanol extracts of *C. manillana* leaves, successfully isolating and identifying six monomeric compounds (Figure 1), all reported here for the first time from this species.

Figure 1. Structures of compounds 1–6

1. Instruments and Materials

Instruments: Bruker Avance-500 NMR spectrometer (TMS as internal standard, Switzerland); Eyela N-1100 rotary evaporator, Eyela SB-1100 water bath, and Eyela N-1100 circulating water vacuum pump (Shanghai Ailang Instrument Co., Ltd., China); Thermo MAT95XP high-resolution electrospray ionization mass spectrometer (Thermo Fisher Scientific, Germany); Hitachi Primaide HPLC system (Hitachi, China); Shimadzu IR Affinity-1 infrared spectrometer (Shimadzu, Japan).

Reagents: Silica gel for column chromatography (60–100, 100–200, 200–300, and 300–400 mesh) and TLC plates were purchased from Qingdao Marine Chemical Factory (China). Reversed-phase silica gel RP-18 (Develosil ODS, 50–70 m) and Sephadex LH-20 dextran gel were from Amersham Biosciences (Sweden). The preparative HPLC column was YMC ODS C18 (20 mm × 250 mm, 5 m). *n*-Hexane, ethyl acetate, acetone, chloroform, methanol, anhydrous ethanol, and concentrated sulfuric acid were obtained from Guangzhou Chemical Reagent Factory and Tianjin Fuyu Reagent Company. HPLC-grade methanol and acetonitrile for preparative chromatography were from Cambridge Isotope Laboratories (CIL, USA).

Plant Material: *C. manillana* var. *longistaminea* leaves were collected from Tianlin County, Baise City, Guangxi Zhuang Autonomous Region (105°50 7 E, 24°22 39 N, altitude 399.1 m) and identified by Professor WEI Xiao from Guangxi Institute of Botany, Chinese Academy of Sciences. The plant habitat and sample processing are shown in Figure 2. The material was air-dried indoors.

Figure 2. Habitat and sample handling of *Champereia manillana* var. *longistaminea*

2. Extraction and Separation

Air-dried *C. manillana* leaves (10 kg) were extracted three times with 95% industrial ethanol at room temperature. The combined extracts were concentrated to yield 1.6 kg of brown crude extract. The extract was suspended in 5 L of pure water and partitioned with *n*-hexane and ethyl acetate until clear layers formed, affording *n*-hexane (600 g) and ethyl acetate (800 g) fractions. The ethyl acetate fraction was mixed with 60–100 mesh silica gel (1:1 ratio), and after solvent evaporation, was subjected to normal-phase silica gel column chromatography with a gradient elution of *n*-hexane/ethyl acetate (v/v 100:0$→$0:1) to obtain eight fractions (Fr. a–Fr. h).

Fraction g (6 g) was separated by reversed-phase silica gel RPC18 using MeOH/H_2O (70%$→100→$1:1) to obtain compound **1** (221.4 mg).

Fraction e (1.4 g) was subjected to RPC18 elution with MeOH/H_2O (70%$→100→$1:1) to obtain compound **3** (43.4 mg).

Fraction d (1.4 g) was separated by RPC18 (MeOH/H_2O, 70%$→100→1 : 1)$ $to yield compound **4** (10.5 mg). Subfraction d3 (52.7 mg) was processed similarly over Sephadex LH-20 to afford two fractions (d3-1, d3-2). Fraction d3-1 (34.3 mg) was purified by normal-phase silica gel (200–300 mesh) with * n * -hexane/ethyl acetate (v/v5 : 1→$1:1) to obtain compound **4** (10.5 mg).

Fraction h (3.3 g) was separated by RPC18 (MeOH/H_2O, 70%$→100→$1:1) to obtain compound **5** (43.2 mg).

Fraction f (3.2 g) was separated by RPC18 (MeOH/H_2O, 70%$→100→$1:1) to give f2-1-1 (32 mg), which was further purified by reversed-phase HPLC (YMC-Pack C18) with 55% acetonitrile gradient elution to yield compound **6** (6.2 mg).

3. Structure Identification

Compound 1 was obtained as white needle-like crystals (chloroform). ESI-MS m/z: 425.3 $[M-H]^-$, $C_{30}H_{50}O$; ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 5.51 (1H, dd, J = 8.3, 3.1 Hz, H-15), 3.17 (1H, dd, J = 11.3, 4.1 Hz, H-3), 2.01 (1H, dt, J = 12.7, 3.1 Hz, H-19), 1.91 (1H, dd, J = 14.4, 2.8 Hz, H-18), 1.07 (3H, s, H-27), 1.00 (3H, s, H-26), 0.93 (3H, s, H-25), 0.92 (3H, s, H-29), 0.91 (6H, s, H-23, 30), 0.82 (3H, s, H-28), 0.80 (3H, s, H-24); ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 158.08 (C-14), 116.87 (C-15), 79.07 (C-3), 55.53 (C-5), 49.28 (C-18), 44.3 (C-9), 41.32 (C-19), 40.0 (C-4), 38.76 (C-8), 37.74 (C-10, 17), 37.57 (C-13), 36.67 (C-16), 35.78 (C-12), 35.12 (C-7), 33.70 (C-1, 21), 33.35 (C-29), 33.09 (C-22), 30.0 (C-26), 29.82 (C-28), 28.80 (C-20), 27.99 (C-23), 27.14 (C-2), 25.90 (C-27), 21.31 (C-30), 18.80 (C-6), 17.50 (C-11), 15.45 (C-24, 25). These data are consistent with literature values (Corbett et al., 1972), identifying compound **1** as taraxerol.

Compound 2 was obtained as reddish-brown needle crystals (ethyl acetate), which appeared orange-red on TLC plates visualized with 10% vanillin-sulfuric acid. ESI-MS m/z: 160.1 $[M-H]^-$, 115.9 $[M-COO]^-$, $C_9H_7NO_2$; ^{1}H-NMR (DMSO-d_6, 500 MHz) δ: 7.17 (2H, m, H-6, 7), 7.42 (1H, brd, J = 7.5 Hz, H-8), 7.99 (1H, brd, J = 7.5 Hz, H-5), 8.01 (1H, s, H-2), 10.9 (1H, s, COOH); ^{13}C-NMR (DMSO-d_6, 125 MHz) δ: 132.69 (C-2), 107.76 (C-3), 121.40 (C-4), 122.55 (C-5), 112.67 (C-6), 121.01 (C-7), 126.45 (C-8), 136.88 (C-9), 166.38 (COOH). Based on literature comparison (Zhang et al., 2009), compound **2** was identified as 1H-indole-3-carboxylic acid.

Compound 3 was obtained as colorless crystals (chloroform). ESI-MS m/z: 461 $[M+H]^+$; $C_{30}H_{52}O_3$; ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 3.34 (1H, m, H-24α), 3.27 (1H, dd, J = 10.5, 3.5 Hz, H-3α), 1.21, 1.15 (each 3H, s, H-26, 27), 0.95 (3H, s, H-18), 0.95 (3H, s, H-28), 0.88 (3H, s, H-30), 0.80 (3H, s, H-29), 0.54 (1H, d, J = 4.0 Hz, H-19), 0.32 (1H, d, J = 4.1 Hz, H-19); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 31.9 (C-1), 30.3 (C-2), 78.8 (C-3), 40.5 (C-4), 47.1 (C-5), 21.1 (C-6), 28.1 (C-7), 48.0 (C-8), 19.9 (C-9), 26.1 (C-10), 26.0 (C-11), 35.9 (C-12), 45.3 (C-13), 48.8 (C-14), 32.9 (C-15), 26.4 (C-16), 52.4 (C-17), 18.1 (C-18), 29.9 (C-19), 36.4 (C-20), 18.4 (C-21), 33.1 (C-22), 28.7 (C-23), 78.8 (C-24), 73.2 (C-25), 23.2 (C-26), 26.5 (C-27), 25.4 (C-28), 14.0 (C-29), 19.3 (C-30). These

data match literature values (Zhou et al., 2009), identifying compound **3** as (24R)-cycloartane-3β,24,25-triol.

Compound 4 was obtained as a colorless solid (chloroform). ESI-MS *m/z*: 439.3 $[M+H]^+$; $C_{31}H_{52}O_2$; ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 3.26 (1H, dd, J = 11.3, 4.4 Hz, H-3), 1.48 (1H, dd, J = 12.0, 4.9 Hz, H-8), 1.24 (1H, dd, J = 11.3, 4.4 Hz, H-5), 0.93, 0.90 (3H, d, H-26, 27), 0.84 (1H, d, J = 6.6 Hz, H-21); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 32.0 (C-1), 30.4 (C-2), 78.8 (C-3), 40.5 (C-4), 47.1 (C-5), 21.1 (C-6), 26.0 (C-7), 48.0 (C-8), 20.0 (C-9), 26.1 (C-10), 26.4 (C-11), 32.9 (C-12), 45.3 (C-13), 48.8 (C-14), 32.9 (C-15), 28.5 (C-16), 52.3 (C-17), 18.1 (C-18), 29.9 (C-19), 36.3 (C-20), 17.9 (C-21), 30.7 (C-22), 28.1 (C-23), 62.8 (C-24), 32.06 (C-25), 19.4 (C-26), 18.3 (C-27), 25.4 (C-28), 14.0 (C-29), 19.3 (C-30), 50.5 (C-31). These data are consistent with literature values (Kuang et al., 2014), identifying compound **4** as (24R,S)-3β-24,31-epoxy-24-methylcycloartane.

Compound 5 was obtained as an oily solid (chloroform). ESI-MS *m/z*: 513.3 $[M+H]^+$; $C_{27}H_{45}O_9$; ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 4.04 (1H, m, H -1), 4.10 (1H, m, H-1), 5.33 (1H, s, H-2), 3.86 (1H, m, H -3), 3.90 (1H, m, H -3); linolenoyl moiety: 2.35 (2H, q, J = 8.4 Hz), 1.63 (2H, m, H-3), 1.31 (6H, s, H-4,5,6), 1.37 (2H, d, J = 7.8 Hz, H-7), 2.08 (2H, m, H-8), 5.36, 5.43 (5H, H-9,10,12,13,15), 5.34 (1H, m, H-16), 2.83 (4H, t, H-11,14), 2.11 (2H, m, H-17), 1.00 (3H, t, J = 7.8 Hz, H-18); D-galactose moiety: 4.31 (1H, d, J = 7.8 Hz, H-1), 3.62 (1H, t, H-2), 3.60 (1H, dd, H-3), 4.02 (1H, s, H-4), 3.57 (1H, t, H-5), 3.88 (1H, dd, H -6), 3.98 (1H, dd, H -6); ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 62.8 (C-1), 69.6 (C-2), 69.7 (C-3); linolenoyl: 173.4 (C-1), 34.1 (C-2), 24.9 (C-3), 29.0, 29.1, 29.2 (C-4,5,6,7), 27.2 (C-8), 127.9 (C-9), 128.2 (C-10), 25.6 (C-11), 131.9 (C-12), 130.2 (C-13), 25.2 (C-14), 128.3 (C-15), 127.8 (C-16), 20.5 (C-17), 14.3 (C-18); D-galactose: 101.1 (C-1), 71.9 (C-2), 73.3 (C-3), 69.6 (C-4), 74.6 (C-4), 62.6 (C-6). Comparison with database literature identified compound **5** as 1-O-linolenoyl-3-O-β-D-galactopyranosyl-sn-glycerol (Kim et al., 2019).

Compound 6 was obtained as a colorless powder (chloroform). ESI-MS *m/z*: 540.5 $[M+H]^+$; $C_{34}H_{68}O_4$; ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 0.85 (3H, t, J = 6.4 Hz, H-31), 1.27 (54H, s, H-4 to H-30), 1.60 (2H, m, H-3), 2.33 (2H, t, J = 7.5 Hz, H-2), 3.60 (1H, dd, J = 11.2, 3.5 Hz, H-3a), 3.70 (1H, dd, J = 11.2, 3.5 Hz, H-3b), 3.92 (1H, m, H-2), 4.37 (1H, dd, J = 11.2, 5.5 Hz), 4.40 (1H, dd, J = 11.2, 3.5 Hz); ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 14.0 (C-31), 22.6 (C-29), 24.8 (C-3), 29.2 (C-4 to C-28), 32.8 (C-30), 34.1 (C-2), 63.4 (C-3), 65.1 (C-1), 70.3 (C-2), 174.3 (C, C-1). These data are consistent with literature values (Nyongha et al., 2010), identifying compound **6** as hyloglyceride.

4. Discussion and Conclusion

This study successfully isolated and purified six monomeric compounds from *C. manillana* leaves using silica gel column chromatography, Sephadex LH-20 gel chromatography, TLC, and HPLC. Based on their physicochemical properties

and spectroscopic data (MS, ^{13}C-NMR, and ^{1}H-NMR), these compounds were identified as taraxerol (1), 1*H*-indole-3-carboxylic acid (2), (24R)-cycloartane-3β,24,25-triol (3), (24R,S)-3β-24,31-epoxy-24-methylcycloartane (4), 1-O-linolenoyl-3-O-β-D-galactopyranosyl-sn-glycerol (5), and hyloglyceride (6). All compounds 1–6 were isolated from this plant for the first time, representing diverse structural classes including terpenoids, fatty acids, and carboxylic acids, thereby providing an initial characterization of the plant' s chemical composition.

Modern pharmacological research demonstrates that terpenoids exhibit antitumor, antimicrobial, antiviral, anti-inflammatory, antihypertensive, and antithrombotic activities (Hai et al., 2015), while fatty acid compounds show anticancer, anti-inflammatory, hepatoprotective, hypoglycemic, and immunomodulatory effects (Ma & Wang, 2006). These properties align with previous reports of *C. manillana* preventing hypertension and cardiovascular diseases (Zhu et al., 2018), suggesting significant potential for pharmaceutical development. However, the specific pharmacological basis and mechanisms of action require further investigation. Future studies could employ bioactivity-guided fractionation to identify active extracts with antihypertensive or hypoglycemic effects, followed by further subdivision to isolate additional bioactive compounds. Additionally, compound **2** (indole-3-carboxylic acid) serves as an important organic intermediate widely used in pharmaceutical and pesticide synthesis (Jiang & Kang, 2015). Previous research has also identified iditol in *C. manillana* (Liu & Xiao, 2009), another pharmaceutical intermediate applicable in chemical industry, cosmetics, and medicine. Therefore, this chemical investigation enriches our understanding of the plant' s material basis and provides a scientific foundation for its future development and utilization.

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Note: Figure translations are in progress. See original paper for figures.